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This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1721177> since 2020-04-03T19:22:26Z

Published version:

DOI:10.1111/jam.14153

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(Article begins on next page)

**Influence of different biological control agents and compost on the rhizosphere and soil total
and nitrification driving microbial communities in a lettuce – *Fusarium oxysporum* f.sp.
lactucae pathosystem**

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28 **Abstract**

29 The response of rhizosphere and bulk soil indigenous total microbial communities focusing on
30 nitrifying prokaryotes was evaluated after the application of different biological control agents
31 (BCAs) (*Bacillus subtilis*, *Trichoderma asperellum* + *Trichoderma gamsii*, *Trichoderma* W2,
32 *Pseudomonas putida*) and compost in controlling lettuce Fusarium wilt. Experiments were
33 conducted “*in situ*” over two lettuce cropping seasons. Total fungal, bacterial and archaeal
34 microbial populations, as well as the ammonia oxidizing groups (i.e., AOB, AOA) and the
35 pathogen, *Fusarium oxysporum* f.sp. *lactucae* (FOL) have been screen out using quantitative
36 polymerase chain reaction (qPCR) method. The BCAs - like communities and different antifungal
37 functional genes (fungal chitinase *chiA*, and bacterial 2,4-diacetylphloroglucinol - *phlD* and HCN
38 synthase - *hcnAB* abundances have been also assessed. Quantitative data were corroborated with the
39 disease index, the potential nitrification activity and chemical parameters of the soil.

40 The application of BCAs and compost resulted in a significantly lower disease severity than the
41 untreated control, and Fusarium wilt was reduced by as much as 60%. This result was confirmed by
42 significant negative correlations between abundance of BCA-like microbial population and disease
43 severity. The BCAs and compost treatments did not negatively affect the microbial communities at
44 the rhizosphere or bulk soil level. The presence of FOL in the untreated control resulted in a niche
45 differentiation of the nitrifiers. Overall, the observed decreased FOL abundance, the increased
46 abundance of inoculum-like populations and the active response of nitrifiers community pointed out
47 the sustainability of the used treatments which achieved an important level of disease suppression.

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50 **Keywords:** BCAs; disease suppression; nitrifying prokaryotes; *chiA* gene, *phlD* gene; *hcnAB* gene

51 **1. Introduction**

52 Lettuce is cultivated and consumed by the whole world and its production exceeded 24 million tons
 53 in 2014 (FAO, 2014). Lettuce crops are grown intensively in Mediterranean regions. Spain, Italy
 54 and France are the three most important producing countries, with 35, 21, and 13%, respectively, of
 55 lettuce production in Europe in 2010 (Eurostat, 2012). *Fusarium oxysporum* f. sp. *lactucae* (FOL) is
 56 a soil-borne, host-specific lettuce pathogen that has been observed in Italy since 2002 (Garibaldi et
 57 al., 2002), which causes serious economic losses (Matheron & Gullino, 2012). Its management has
 58 been investigated intensively through different methods, such as solarization (Matheron and
 59 Porchas, 2010) or the application of different organic amendments (Franceschini et al., 2016;
 60 Gilardi et al., 2016). However, the seed transmission of this pathogen makes soil disinfection only
 61 partially effective (Garibaldi et al., 2004; Gilardi et al., 2007). For these reasons, the use of
 62 biological control agents (BCAs), based on antagonistic microorganisms or different composts (e.g.,
 63 compost with inoculated BCA, green compost), is considered an effective and sustainable strategy
 64 (Barrière et al., 2014; Pugliese et al., 2015). Species of the *Trichoderma* genus (*T. harzianum*, *T.*
 65 *viridi*) have been shown to be efficient “biofungicides”, as they are involved in the suppression of
 66 different pathogens, including *Fusarium oxysporum* f. sp. *lactucae* (Gilardi et al., 2007, 2013;
 67 Innocenti et., 2015). *Pseudomonas* spp. is known to be responsible for the “natural” suppressiveness
 68 of some soils against soil-borne pathogens (e.g., *Pythium ultimum*, *Rhizoctonia solani*) (Weller et
 69 al., 2002; 2007), and the same phenomenon has also been observed for various *Bacillus* species
 70 (e.g., *B. subtilis*, *B. thuringiensis*, *B. amyloliquefaciens*) (Cawoy et al., 2011; Kloepper et al., 2004).
 71 Many studies have been conducted on the effects of BCAs and compost on disease control
 72 (Bonanomi et al. 2007; Berg, 2009; Pane et al. 2013). The presence of BCAs candidates in compost,
 73 belonging to the *Trichoderma*, *Pseudomonas* and *Bacillus* genera, has also been demonstrated
 74 (Pugliese et al., 2008).
 75 BCAs are known to be aggressive colonizers that indirectly affect the functional guilds in the
 76 rhizosphere environment (Gupta et al., 2012). However, their introduction into a new soil system is
 77 a complex process which may disturb the indigenous soil microorganisms that play crucial roles in

78 the essential nutrient cycles, especially nitrogen (N) cycle. A critical component of the microbially
79 driven N cycle is the nitrification process, encoded by *amoA* as key genes that controls the
80 availability of soil nitrates (Nicol et al., 2008; Zhang et al., 2013). N supply and form affect lettuce
81 plant defense and FOL pathogenicity (Orr and Nelson, 2018). Nevertheless, little information are
82 available regarding the impact of BCAs and compost may have on ammonia-oxidizing bacteria or
83 on the archaea that harbor the *amoA* gene (AOB and AOA) (Musyoki et al., 2014; Zimmermann et
84 al., 2016), and in consequence on the soil N form which may further control FOL suppression.
85 Hence, there is a critical need to study the potential side - effects of the applied treatments on both
86 AOB and AOA.

87 The primary objective of this study was to explore the direct impact of BCAs and compost
88 treatments “*in situ*” on the abundance of total fungi, bacteria and archaeal communities, as well as
89 on the ammonia oxidizing groups (i.e., AOB, AOA) in the FOL affected lettuce rhizosphere and
90 bulk soil. Moreover, fungal and bacterial recruitment upon pathogen infection, has been assessed by
91 studying three anti-pathogen genes that are usually correlated with disease suppression: the
92 *Trichoderma* based chitinase *chiA* gene, and two well-described bacterial antifungal genes: *phlD*
93 (coding for 2,4-diacetylphloroglucinol - 2,4-DAPG) and *hcnAB* (coding for hydrogen cyanide -
94 HCN).

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103 2. Materials and Methods

104 2.1. Layout of the experiments

105 The experiments were carried out in tunnels (6 x 60 m) on a commercial farm in Moretta (Cuneo,
106 Northern Italy), which had a history of several lettuce cycles prior to the beginning of this study.
107 The soil was characterized as a silty loam soil (silt : sand : clay 60 : 30 : 10 %, with neutral pH and
108 1.5 % organic matter) naturally infested with the FOL pathogen.

109 The lettuce cultivar ‘Novelsky’, which belongs to the batavia type and is moderately susceptible to
110 Fusarium wilt was used (Gilardi et al., 2017). The experimental trials were carried out over two
111 years, 2016 and 2017, in order to test the efficacy of pre-planting treatments with different
112 commercially available *Bacillus subtilis*: Serenade Max – SM, QST713, Bayer Crop Science, Italy
113 and *Trichoderma gamsii* + *Trichoderma asperellum*: Remedier – RM ISAGRO, Italy) and
114 experimental BCAs (*Trichoderma*, TW2, AgriNewTech, Italy and *Pseudomonas putida* - Pp,
115 Agroinnova, Italy) (Table 1) against lettuce wilting. In addition, two composts (Ant’s Compost V
116 and Ant’s Compost M; AgriNewTech, Italy), produced from green wastes, in a dynamic industrial
117 treatment system, were used (Table 1): one green compost - CV and the same compost inoculated
118 with *Trichoderma* TW2 - CM. An untreated control was used to monitor Fusarium wilt
119 development. Summarized details of these treatments can be found in Table 1.

120 Lettuce seeds were sown in 100-plug trays (3.4 cm diameter pots, 4 l of soil, 53 x 42 cm surface)
121 filled with a peat mixture substrate.

122 Four substrate (i.e., peat) treatments with BCAs were carried out on the plug tray between sowing
123 and transplanting (at the first true leaf stage) in a commercial nursery; incubation until transplanting
124 was conducted under greenhouse conditions at a temperature of 22-24°C (Table 1). The BCAs were
125 applied by spraying them onto the peat surface in a high volume of water (500 ml/tray) using a 1 l
126 capacity hand sprayer. The compost products were distributed in a thin layer of 8 g/plug at sowing
127 (T0) over the surface of 53 x 42 cm plug trays, then mixed with the soil immediately before
128 transplanting (T20). Azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection, Italy) was used as
129 the reference chemical treatment and was applied once as a soil drench at transplanting.

130 Twenty day old lettuce plants were transplanted (at T20) at a density of 24 plants/m², drip irrigated
131 and grown according to the cultural practices adopted by commercial growers in the region. Each
132 treatment had four biological replicates arranged in a completely randomized block design. The
133 plants were monitored weekly for symptoms development, and the data were recorded starting from
134 the appearance of the first symptoms (yellow leaves and reduced growth). The final disease rating
135 was carried out 48-53 days after transplanting on 16 plants/treatment by dissecting each plant. The
136 disease severity (DS) scale was as follows: 0 = healthy plant, 25% = initial leaf chlorosis, 50% =
137 severe leaf chlorosis and initial symptoms of wilting during the hottest hours of the day, 75% =
138 severe wilting and severe symptoms of leaf chlorosis; 100% = plant totally wilted, leaves
139 completely necrotic. Lettuce yield was measured at the end of the trials by collecting 12 plants per
140 treatment, and determining the fresh weight of the plants.

141

142 2.2. Rhizosphere and bulk soil sampling

143 The influence of soil-treatments with BCAs and compost on indigenous microbial communities was
144 studied at the rhizosphere and bulk soil levels by collecting samples at the end of the trials (the end
145 of September 2016 and 2017). One composite rhizosphere sample per plot consisted of the roots of
146 five randomly selected lettuce plants. The roots were shaken vigorously to remove the soil particles
147 that were not tightly adhering on them. The rhizosphere soil was gently scraped off with sterile
148 forceps and transferred into sterile sampling bags. Each treatment consisted of three biological
149 replicates, which were kept on ice. Soil bulk samples were taken also in three replicates (0-15 cm
150 soil depth) per treatment. Each replicate consisted of five sub-replicates collected from the same
151 plots as the rhizosphere samples. The replicates were sampled in a W - shaped sampling pattern, to
152 obtain a good bulk soil representation and homogeneity. The samples were then placed in plastic
153 bags, kept on ice and transported to the laboratory.

154 The fresh rhizosphere and bulk soil samples were homogenized, passed through a 2 mm sieve and
155 stored at 4°C. All the samples were split into two parts: one part was sent to the Regional Chemistry

156 Laboratory (Turin, Italy) for further geochemical analysis: humidity, pH, total N (TN), inorganic N
157 as nitrate (NO_3^-) and ammonium (NH_4^+), total organic carbon (TOC), total phosphorus (TP) and
158 available phosphorus (AP). The second part was used for enzymatic measurements (i.e., potential
159 nitrification activity – PNA) and further microbiological and molecular investigations.

160

161 2.3. Molecular analyses

162 2.3.1. Rhizosphere and bulk soils DNA extraction

163 A NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used,
164 according to the manufacturer's instructions, for the rhizosphere and bulk soil genomic DNA
165 extraction. Extraction was conducted on fresh samples (250-500 mg of sample material). DNA
166 quantity and purity were measured spectrophotometrically using a NanoDrop ND-1000 (NanoDrop
167 Technologies, Wilmington, DE, USA).

168

169 2.3.2 Microbial abundance (quantitative PCR (qPCR) assays)

170 The abundance of the bacterial and archaeal 16S rRNA genes, fungal 18S rRNA genes and of five
171 functional genes (i.e., bacterial ammonia monooxygenase *amoA* gene - AOB, archaeal *amoA* gene -
172 AOA, fungal chitinase *chiA* gene, and bacterial 2,4-diacetylphloroglucinol *phlD* and HCN synthase
173 *hcnAB* genes) was determined by means of qPCR, using a StepOnePlus™ Real-Time PCR System
174 (Applied Biosystems, Foster City, CA, USA) for the rhizosphere and bulk soil DNA samples. In
175 addition, the abundance of FOL and BCAs - like microorganisms (i.e., *Bacillus*, *Trichoderma* and
176 *Pseudomonas*) was also investigated. A description of the primer sets and amplification details are
177 given in Table 2.

178 The primers for fungal chitinase *chiA* gene quantification were designed as follows: DNA from
179 rhizosphere and bulk soil samples from the treatments with *Trichoderma* sp. was used to amplify
180 the fungal chitinase *chi42* gene, according to the Nguyen HL et al., (2001) protocol, with
181 CHIf/CHIr primers, which generally amplify a 1450 bp sequence. Four new primer pairs targeting

an internal fragment of the *chi42* gene, were designed using Gen Script (<http://www.genscript.com/tools.html#biology>), considering relatively high annealing temperatures (≥ 60 °C) and smaller amplicon sizes than 200 bp to reduce generation of artifacts and to achieve appropriate reaction efficiencies during qPCR. The accuracy of the primers was confirmed using BLAST of the National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>); preliminary tests (data not shown) were carried out to assess their specificity, and the best primer pair was then chosen. The designed primer pair (chiaxF (ACCCTGCCGATGACACTCAG)::chiaxR (GGCAGCGATGGAGAGAAGGA) generated an amplicon of 122 bp, with the following sequence:
5'ACCCTGCCGATGACACTCAGGCCACCAACATGGTTCTTCTGCTCAAGGAGATCCGAT
CTCAACTAGATGCCTATGCTGCGCAATACGCTCCAGGCTACCACTTCCTTCTCTCCATC
GCTGCC-3'.

Amplicons from each target gene were generated for the standard preparation, purified (Invisorb Fragment CleanUp, Stratec Molecular GmbH, Berlin, Germany), and ligated in Strata-Clone PCR cloning vector pSC-A (Strataclone PCR Cloning Kit, Agilent Technologies Inc.); the ligation products were then transformed into StrataClone SoloPack competent cells (Agilent Technologies Inc.). The specificity of the clones used as qPCR standards was checked via sequencing, at LGC Genomics GmbH (Berlin, Germany) and through BLAST analysis. Plasmid DNA was isolated (GenElute™ Plasmid Miniprep Kit, Sigma-Aldrich, St. Louis, MO, USA) from standard clones and quantified as described above.

As assessed in preliminary tests and in a qPCR assay optimization, the final volume of the qPCR reaction cocktails was 25 µl for the 16S and 18S rRNA genes, the bacterial and archaeal *amoA* gene, and the FOL and BCA - like microorganisms quantification, and it was 20 µl for the *chiaA*, *phlD* and *hcnAB* genes. All the quantifications were conducted with a 10 ng DNA template, except for the bacterial 16S rRNA gene, for which a 5 ng DNA template was used (Cucu et al., 2017). The reaction mixtures contained 1x Power SYBR green master mix (Applied Biosystems), 0.12 µM of

208 each oligonucleotide (Table 2) for the total bacteria, archaea, fungi and FOL, 0.15 μM for the *phlD*
209 and *hcnAB* genes, 0.32 μM for the AOB, AOA and BCA - microorganisms and 0.4 μM for the *chiA*
210 gene.

211 All the considered genes were quantified in triplicate across plates, while standards were run in
212 duplicate in 10-fold serial dilutions. The optimal dilution of the DNA extracts was tested to
213 compensate for any inhibitory reactions by the organic compounds (e.g., humic acids) co-extracted
214 during the DNA isolations. In all experiments, negative controls containing no-template DNA were
215 subjected to the same qPCR procedure to detect and exclude any possible contamination.
216 Amplification efficiency ranged from 96%, (archaeal 16S rRNA, fungal 18S rRNA, BCAs and their
217 functional genes) to 103% (bacterial 16S rRNA). For the nitrifiers, the amplification efficiency
218 were 95.3% and 99.1% for AOB and AOA, respectively. The R^2 was always ≥ 0.98 . Melting curves
219 of the amplicons were generated to ensure that the fluorescence signals originated from specific
220 amplicons and not from primer dimers or other artifacts. This was confirmed by checking the
221 amplification products on 1% agarose gel. Gene copy numbers were calculated with StepOne™
222 software, version 2.2 (Applied Biosystems). The data were normalized and presented as log copies
223 g^{-1} dry soil.

224

225 2.4. Chemical properties of rhizosphere and bulk soil samples

226 In short, the pH values were measured in water suspensions at a solid: liquid ratio of 1:2.5. The total
227 organic carbon (TOC) and total nitrogen (TN) were quantified using a Leco Tru Spect CN
228 automatic analyzer. Ammonium (NH_4^+) and nitrate (NO_3^-) were measured colorimetrically, by
229 means of a continuous flow auto-analyzer (Alliance Evolution II), using standard colorimetric
230 techniques. The total phosphorus (TP) was determined by means of 'ICP Varian mod. Liberty LR',
231 after microwave digestion with hydrogen peroxide, hydrochloric acid and nitric acid, filtration and
232 dilution. The available P (AP) was extracted using sodium bicarbonate and determined by means of

233 the molybdenum blue method (Olsen et al., 1954), modified for continuous flow colorimetric
234 analysis (Alliance Evolution II).

235 The potential nitrification activity (PNA) was determined according to the procedures described by
236 Hoffmann et al. (2007).

237

238 2.5. Statistical analyses

239 The data on disease index, gene abundance, chemical properties of the soil and potential
240 nitrification activity were subjected to a linear mixed model, with year considered as the random
241 variable, using R software (Software R 3.0.1, R foundation for Statistical Computing, Vienna,
242 Austria, <http://www.R-project.org>). The effects of different BCAs and compost on the abundance of
243 the studied genes, on the chemical properties of the soil and on disease severity were evaluated. All
244 data were subjected to a Levene test to check for the homogeneity of variance and normality was
245 tested on the residuals using the Shapiro-Wilk test; when not normally distributed data from disease
246 severity (DS) were arcsin transformed while, the data from microorganisms abundance were log-
247 transformed and normality was checked again. The means were separated by Bonferroni test. The
248 statistical analysis included treatment rhizosphere soil, bulk soil, year, treatment \times year.

249 Pearson's linear correlation coefficients were calculated for assessing the relations between
250 microbial gene abundance, disease severity and PNA, as well as between *Pseudomonas*, *Bacillus*,
251 *Trichoderma* and FOL abundance and soil chemical properties.

252 Linear regression between total microbial gene abundance and the functional genes of N cycle
253 (bacterial *amoA*, archaeal *amoA*, *chiA* gene) with soil chemical data were calculated to evaluate how
254 much of their abundance is explained by variation in the soil chemical data.

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258 3. Results

259 3.1. Disease severity and plant biomass

260 The lettuce plants in the non-treated control plots showed an average disease severity DS of up to
261 60% at the end of both trials (Table 3). The BCA treated plots provided significantly lower disease
262 severities than the untreated ones, and statistically similar results to one application of azoxystrobin,
263 reducing Fusarium wilt by 47.2 to 58.5% in the two years, respectively. As a consequence, the fresh
264 weight of the lettuce was significantly higher in all the treatments compared to control, without
265 statistically differences among them (Table 3). No significant difference was observed between the
266 disease severity data. A significant time effect for fresh weight of the lettuce between trials was
267 noted ($p < 0.001$). However, the *Pseudomonas* and *Trichoderma* treatments were less effective in
268 the second trial.

269

270 3.2. Microbial abundance

271 3.2.1. FOL abundance

272 In general, FOL abundance assessed by gene copy quantification was significantly higher in the
273 bulk soil than in the rhizosphere soil. Almost all the treatments resulted in a significant reduction of
274 pathogen - associate gene copies compared to the untreated control - C, in both the rhizosphere and
275 bulk soil at the end of both trials (2016 and 2017 respectively) (Table 4). At the end of first trial
276 (2016), Serenade max - SM - *Bacillus* treatment was not significantly different compared with the
277 untreated control - C at the rhizosphere level, while at the end of the second trial (2017) the
278 *Pseudomonas* - Pp treatment was not significantly different compared with the untreated control –
279 C. The interaction treatment \times year was not significant (Table 4).

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284 3.2.2. Total microbial population abundance

At the end of both trials (2016 and 2017 respectively) the number of gene copy of bacterial, archaeal and fungal populations showed an important homogeneity, with no significant interaction treatment \times year. Therefore the data were presented as average of all values from both trials.

Overall, at the end of both trials (2016 and 2017 respectively) the bacterial 16S rRNA abundance was significantly higher at the bulk soil level than at the rhizosphere level (Figure 1a). The effect of BCAs treatments was more evident in the rhizosphere; the Remedier - RM - *Trichoderma* spp. and *Pseudomonas* - Pp resulted in a lower bacterial abundance than the other treatments but not significantly different than the untreated control - C, while the Serenade max - SM - *Bacillus* treatment resulted in a significantly higher bacterial abundance than the untreated control - C. However, the bacterial community abundance was more or less stable at the soil level over all the treatments (Figure 1a). The archaeal 16S rRNA gene copy number in the rhizosphere followed almost the same trend as the overall bacterial counterpart. The green compost - CV and *Trichoderma* W2 - TW2 treatments resulted in slightly higher archaeal abundance in the bulk soil samples than the untreated control - C (Figure 1b). The fungal 18S rRNA abundance was significantly higher in the rhizosphere than in the bulk soil samples, in all the treatments as well as in the untreated control - C. Remedier - RM - *Trichoderma* spp. inoculation resulted in a slightly higher fungal abundance than the untreated control - C, while the total fungal community in the soil samples was more abundant in the Serenade max - SM - *Bacillus*, *Pseudomonas* - Pp and green compost - CV treatments (Figure 1c).

3.2.3. Functional gene abundance

In general, the ammonia-oxidizing bacterial (AOB) gene abundance was significantly influenced by treatments and was significantly higher in the bulk soil than in the rhizosphere samples (Table 5). After the first trial, 2016, the AOB gene in the bulk soil samples was more abundant in the untreated control - C, while the lower abundance was observed after Serenade max - SM - *Bacillus* treatment. At the rhizosphere soil level the Serenade max - SM - *Bacillus*, green compost - CV and

311 *Trichoderma* W2 – TW2 treatments resulted in a highest AOB gene abundance while no significant
312 difference was observed between Remedier - RM-*Trichoderma* spp., *Pseudomonas* – Pp and the
313 untreated control - C. After the second trial, 2017, the same trend of AOB abundance was observed
314 at the bulk soil level, while at the rhizosphere soil level the Remedier - RM-*Trichoderma* spp. and
315 *Pseudomonas* – Pp treatments resulted in the lowest AOB abundance. The interaction treatment \times
316 year was not significant. (Table 5).

317 In general, the ammonia-oxidizing archaeal (AOA) gene abundance was significantly influenced by
318 treatments and was significantly higher in the bulk soil than in the rhizosphere soil samples (Table
319 5).

320 All the treatments resulted in higher archaeal *amoA* gene abundance than the untreated control - C,
321 at both rhizosphere and bulk soil levels for both trials, 2016 and 2017 respectively. After the first
322 trial, 2016, the green compost - CV treatment resulted in the highest AOA abundance than the other
323 treatments at the bulk soil level, while the Serenade max - SM – *Bacillus* and *Trichoderma* W2 –
324 TW2 treatments resulted in the highest abundance than the other treatments at the rhizosphere soil
325 level. After the second trial, 2017, the green compost - CV and the Remedier - RM-*Trichoderma*
326 spp treatments resulted in the highest AOA abundance at the bulk soil level, while the Serenade
327 max - SM – *Bacillus* treatment resulted in the highest AOA abundance at the rhizosphere level. The
328 interaction treatment \times year was not significant.

329 In general, the *chiA* gene (*Trichoderma* based chitinase) abundance was significantly influenced by
330 treatments (Table 6). After the first trial, 2016, the abundance of the *chiA* gene was higher in the
331 rhizosphere soil samples for the *Trichoderma* TW2 – TW2 and Remedier - RM-*Trichoderma* spp
332 treatments. However the Remedier - RM-*Trichoderma* spp was not statistically different compared
333 with the inoculated compost – CM. The *Pseudomonas* - Pp and the green compost - CV treatments
334 resulted in the lowest *chiA* gene abundance compared with the other treatments, but not
335 significantly different compared with the untreated control - C. At the bulk soil level, the *chiA* gene
336 was more abundant in the Remedier - RM-*Trichoderma* spp, *Pseudomonas* - Pp and green compost

337 - CV treatments comparing with the other treatments and with the untreated control - C (Table 6).

338 After the second trial, 2017, all the treatments at the rhizosphere soil level resulted in significant

339 differences between the *chiA* gene abundances. The highest *chiA* gene abundance was observed in

340 the *Trichoderma* W2 - TW2 treatment. Also at the bulk soil level all the treatments resulted in

341 higher *chiA* abundance compared with the untreated control - C. The highest *chiA* gene abundance

342 was observed in the *Pseudomonas* - Pp and green compost - CV treatments.

343 The abundance of the *phlD* gene was significantly higher in the rhizosphere than in the bulk soil

344 samples for all the treatments and for the untreated control - C (Table 6). After the first trial, 2016,

345 *Pseudomonas* - Pp and the green compost - CV treatments resulted in the highest *phlD* abundance at

346 the rhizosphere soil level, while the same treatments resulted in the lowest *phlD* abundance at the

347 bulk soil level. After the second trial, 2017, *Pseudomonas* - Pp treatment resulted in the highest

348 *phlD* abundance at the rhizosphere soil level. At the bulk soil level, the highest abundance was

349 observed after *Trichoderma* W2 - TW2 treatment, while *Pseudomonas* - Pp and the green compost

350 - CV treatments resulted in a lower *phlD* gene abundance than the untreated control - C.

351 The abundance of the *hcnAB* gene was significantly influenced by treatments and it was

352 significantly higher in the rhizosphere than in the bulk soil samples for all the treatments and for the

353 untreated control - C (Table 6). After the first trial, 2016, the highest *hcnAB* gene abundance was

354 observed after *Pseudomonas* - Pp , inoculated compost – CM and *Trichoderma* W2 – TW2

355 treatments at the rhizosphere level. At the soil level the *hcnAB* gene abundance was not

356 significantly different compared with the untreated control –C, with the exception of *Pseudomonas*

357 - Pp and the green compost - CV treatments which resulted in the lowest *hcnAB* gene abundance.

358 After the second trial, 2017, the *hcnAB* gene abundance was highest after the inoculated compost -

359 CM treatment and in the untreated control - C was noted the lowest *hcnAB* gene abundance at the

360 rhizosphere level. At the soil level the *hcnAB* gene abundance was higher in the untreated control C

361 and the *Trichoderma* W2 – TW2 treatment while the lowest *hcnAB* gene abundance was noted after

362 *Pseudomonas* – Pp and the green compost – CV treatments.

363

364 3.2.4. BCAs - like microorganisms abundance

365 Overall, at the end of both trials (2016 and 2017 respectively) the BCA applications resulted in an
366 increase in the indigenous populations of *Bacillus*, *Trichoderma* and *Pseudomonas* in all the
367 treatments, compared to the untreated control - C (Table 7). No significant interaction treatment \times
368 year was observed. In general, the abundance of *Bacillus* sp. was significantly higher for the
369 *Bacillus* - SM treatment than the untreated control - C and all the other treatments in both the
370 rhizosphere and bulk soil. However, the inoculated compost treatment - CM also resulted in an
371 increased resident *Bacillus* abundance in rhizosphere and bulk soils after both trials. Nevertheless,
372 the *Trichoderma* TW2 - TW2 treatment showed similar levels to those of the untreated control - C.
373 Significant interactions bulk \times rhizosphere were observed (Table 7).

374 The inoculation with the *Pseudomonas* - Pp significantly increased the overall *Pseudomonas*
375 community in both the rhizosphere and bulk soils, compared to the untreated control - C and the
376 other treatments. Significant interactions bulk \times rhizosphere were observed (Table 7).

377 The inoculation with the *Trichoderma* - based formulate significantly increased the overall
378 *Trichoderma* community in both the rhizosphere and bulk soils, compared to the untreated control -
379 C. After both trials, the highest abundance of *Trichoderma* was observed in the rhizosphere after
380 *Trichoderma* TW2 - TW2 treatment and in the bulk soil after the green compost – CV treatment.
381 After the second trial, 2017, a highest *Trichoderma* abundance was also observed after the
382 inoculated compost - CM treatment at the bulk soil level. The Serenade max - SM – *Bacillus*
383 application resulted in somewhat lower *Trichoderma* abundance than all the other BCA
384 applications. Significant interactions bulk \times rhizosphere were observed (Table 7).

385

386

387 3.3. Chemical properties

388 Generally for both trials, 2016 and 2017 respectively, pH was significantly lower ($p < 0.05$) at the
389 rhizosphere level than at the bulk soil level, with values ranging from between 5.70 and 6.43 for the
390 rhizosphere and between 6.34 and 6.77 for the bulk soil samples. The concentrations of TN, NH_4^+ ,
391 NO_3^- and TOC were generally significantly higher in the rhizosphere than in the bulk soil samples.
392 The untreated control - C was characterized by higher NH_4^+ , NO_3^- and TOC concentrations than all
393 the treatments. On the other hand, the highest TP and AP contents were observed in the bulk soil
394 samples (Table S1). No significant treatment \times year interaction was found.

395

396 3.4. Potential Nitrification Activity (PNA)

397 The PNA measurements generally exhibited significantly higher values at the bulk soil level than in
398 the rhizosphere level. No significant treatment \times year interaction was observed, therefore the data
399 were presented as the average of all data from both trials, 2016 and 2017 respectively. The PNA in
400 the bulk soil was significantly ($p < 0.05$) higher in the untreated control - C, followed by the
401 inoculated compost - CM and the *Pseudomonas* - Pp treatments. The lowest value was observed
402 after both, Serenade max - SM – *Bacillus* and Remedier - RM-*Trichoderma* spp treatments. The
403 PNA in the rhizosphere was significantly lower ($p < 0.05$) in the untreated control - C than in the
404 other treatments. (Figure 2).

405

406 3.5. Correlations between the levels of total microbial communities (16S bacteria, 16S archaea, 18S
407 fungi), the functional genes (fungal *chiA* gene, bacterial and archaeal *amoA* genes, bacterial *phlD*
408 and *hcnAB* genes), the *Bacillus*, *Pseudomonas*, *Trichoderma* population levels and FOL
409 abundances, DS and PNA.

410 In general the correlations were negative, with r coefficients ranging from -0.37 to -0.5 ($p < 0.001$)
411 at the rhizosphere level and from -0.3 to -0.6 ($p < 0.001$) at the bulk soil level (Table 8). Very
412 strong positive correlations were observed between FOL and DS, as well as between the
413 nitrification rate - PNA and DS. Strong negative correlations were recorded at the rhizosphere and

bulk soil levels especially between AOB and DS at the soil level, and between AOA and PNA at the soil level (Table 8).

3.6. Correlations between *Bacillus*, *Pseudomonas*, *Trichoderma* population levels and chemical properties in rhizosphere (rhizo) and soil samples

In general the correlations were significant only with respect to AP, TP, NO_3^- and TOC, with r values ranging from -0.5 to 0.8 (Table 9).

3.7. Regressions between indigenous microbial communities and soil chemical properties

Variance in the total bacterial abundance was explained by alterations of NO_3^- and TP at the rhizosphere level (Table S2). Changes in the total archaeal abundance were explained by alterations of NO_3^- and TP at the rhizosphere and soil level respectively. Changes in the total fungal community were explained by alterations of TP and TOC at the rhizosphere level and TP at the soil level. The variance in *chiA* gene abundance was explained by alteration of TN including NO_3^- and NH_4^+ at both rhizosphere and soil level. AOA abundance changes were explained by TN, NO_3^- and TOC at the rhizosphere level and by NO_3^- at the soil level. However, a greater percentage of AOB was explained also by NO_3^- (Table S2).

4. Discussion

In the present study, rhizosphere and soil microbial community changes have been investigated monitoring total bacterial, archaeal and fungal highly distinctive genes abundance as indicators of the general alterations that may take place as a result of plant treatments and the infection of lettuce by FOL. In this context, the potential impact of various BCA and compost treatments on the total indigenous microbial communities and on the functional genes that controls nitrogen availability was assayed.

4.1. Disease severity, FOL abundance and plant biomass

441 The obtained results were in line with previous studies showing that the application of BCAs and
442 certain organic matter can effectively suppress soil pathogens (Zhang et al., 2008; Luo et al., 2009;
443 Wu et al., 2009; Ling et al., 2010; Cao et al., 2011; Lang et al., 2011; Yang et al., 2011; Gilardi et
444 al., 2016). The application of different BCAs and compost significantly reduced wilting by
445 suppressing FOL abundance compared to the untreated control. This result was corroborated by the
446 significant positive correlations ($p < 0.001$) that were observed between disease severity and FOL
447 abundance. These findings were supported by previous studies that showed that *Fusarium*
448 abundance in the cucumber and banana rhizosphere was reduced after BCA treatments (Qiu et al.,
449 2012; Shen et al., 2015; Fu et al., 2017). Furthermore, FOL abundance was very low after the
450 treatment with inoculated compost (CM), thus indicating that an effective disease control could be
451 achieved with organic amendments, as previously pointed out (Bonanomi et al., 2010). Similar
452 results were obtained in a previous study (Pugliese et al., 2011), confirming that inoculation of
453 compost with *Trichoderma* strains can improve the suppressive activity of the substrate. The
454 application of compost may have increased the competition for resources and the interactions
455 between antagonist species of compost and soil, and this may have resulted in a reduction in the
456 FOL abundance and in the potential activity of the pathogen (Bonilla et al., 2012; Larkin et al.,
457 2015).

458

459 4.2. Ecological fitness of the BCA - like microorganisms and their effect on the total indigenous 460 prokaryotes

461 Our results have evidenced an excellent feedback of the BCA - like microorganisms after the BCAs
462 were introduced into the soil, as inoculated plantlets or inoculated compost. This was highlighted by
463 significant negative relationships ($p < 0.001$) between the BCA – like populations and *Fusarium*
464 wilt severity, which suggested not only the ecological fitness of inoculated microorganisms, but
465 also their efficiency against FOL. Most probably, the efficiency of biological control of FOL was
466 based on mechanisms as saprophytic competition for nutrients, parasitic competition for infection

467 sites and induced resistance in host cells (Haas and Defago, 2005; Nel et al., 2006; Srinivasan et al.,
468 2009). Even more, the good relationships between the BCAs - like microorganisms and soil
469 nutrients highlighted the role of the antagonists in solubilising certain compounds (nitrogen,
470 phosphorus, potassium, iron) (Gyaneshwar et al., 2002; Richardson et al., 2009) contributing to the
471 disease suppression.

472 The application of BCA - based formulates resulted in a subsequent boost in abundance of the
473 respective microbial populations (i.e., *Pseudomonas*, *Trichoderma* and *Bacillus*). A remarkable
474 result of this study concerns the high abundance that was observed in the rhizosphere samples of the
475 *phlD* and *hcnAB* genes known to encode the production of antifungal compounds such as 2,4-
476 diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN) (Dudenhoffer et al., 2016). We
477 speculated that *Pseudomonas spp* (e.g., *P. fluorescens*) abundance would have increased in the
478 rhizosphere environment upon pathogen infection, as these bacteria are strong root colonizer
479 (Looper et al., 2012). This may explain the lower observed abundance of the total *Pseudomonas*
480 community compared with other treatments. This was probably due to the action of antifungal
481 metabolites against FOL, but also against other microorganisms that generally colonize the
482 rhizosphere. The *chiA* gene abundance confirmed the potential antagonistic activity of the
483 inoculated *Trichoderma* strain TW2, but also of the inoculated compost. In addition, a significant
484 relationship of *chiA* gene abundance with the total and especially with ammonium N ($p < 0.001$)
485 content was observed (Table S2). This result highlighted the involvement of fungal population in
486 remineralization of nutrients during the decomposition of chitin (Gould et al., 1981) with important
487 contribution to nitrification process.

488 Overall, we did not observe any negative side-effects of BCA application on the total microbial
489 communities, thus indicating that the microorganisms introduced into the soil system may have
490 favoured the resident microbial populations through positive interactions. Our results were in line
491 with previous reports (Ghini et al., 2000; Gullino et al., 1995; Mezzalama et al., 1998). Contrary to
492 our expectations, a higher total fungal abundance was observed in the rhizosphere than in the bulk

soil. The boost in the fungal community in all the treatments and in the untreated control was probably supported by the elevated level of organic carbon (Table S1). This may be linked to a competitive potential, based on an increased root exudation, which favours rhizosphere colonization by fungi, including FOL. In this context, Strange (2005) showed that fungi mobilize nutrients through the production of growth regulators. This may explain the observed positive correlation between AP and the total fungal community in both the rhizosphere and bulk soil. On the other hand, a lower archaeal population level was observed in the rhizosphere, probably as a result of the apparently lower pH. Moreover, the archaeal community may also have been lower due to a lower growth rate and competitiveness than the bacteria and fungi (Karlsson et al., 2012). In addition, in the present study, the archaeal community was negatively correlated ($p < 0.05$) with the disease severity, thus suggesting a likely competition for nutrients with FOL. This may underline the possible ability of archaea to contribute to the suppression of lettuce Fusarium wilt development. In a previous study, Mendez et al. (2013) described a relationship between archaeal community composition and soil suppressiveness to *Rhizoctonia* damping-off. However, further investigations are needed to clarify whether archaea play an important role in the effective protection of lettuce against FOL.

509

4.3. Ammonia oxidizing archaea and bacteria

The archaeal nitrifiers (AOA) were more abundant in the soil samples than their bacterial counterparts. In fact, a lower AOA abundance was observed in the rhizosphere than in the bulk soil, which may be a result of a high organic carbon content and a lower pH (Wessén et al., 2010; Bates et al., 2011). In addition, a low ammonia environment represents the key factor that determines a niche separation of AOA and AOB in acidic/neutral soils. A strong positive relationship was observed between the AOB and TN at the rhizosphere level, and this resulted in a dominance of bacterial nitrifiers and their resilience to lower pH compared to AOA (Wessén et al., 2010). The result supports the finding of Valentine (2007), who showed that all archaea, and their nitrifiers

519 components in particular, in general have a higher competitive advantage under reduced organic
520 resource conditions. Accordingly, we observed a higher AOA abundance in the soil samples, which
521 indicated that the archaeal nitrifiers, and in general the entire archaeal community, were probably
522 adapted to the decomposition of recalcitrant organic matter, as described earlier on by Cucu et al.,
523 (2017).

524 Overall, the effect of different applied treatments resulted in the increase of nitrifiers abundance
525 with respect to the untreated control thus suggesting that BCAs may not have negative ecological
526 impacts on other groups of microorganisms. Comparing with all treatments,, a higher AOB
527 abundance was observed in the bulk soil of the untreated and FOL infested control, while the
528 opposite was true for the AOA in the rhizosphere, thus suggesting that the sole presence of FOL
529 may has induced a differentiation, in the rhizosphere and bulk soil, among the indigenous
530 prokaryotes involved in the nitrification process. Hence, the AOB abundance was not reduced in the
531 control samples, probably as a result of a niche differentiation among nitrifiers at the rhizosphere
532 and bulk soil levels induced by the FOL presence, rather than the commonly acknowledged direct
533 resource competition with AOA (Nicole et al., 2008; Wessén et al., 2010; Musyoki et al., 2015).
534 Additionally, a strong negative relationship between the nitrification rate and disease severity was
535 observed at the bulk soil level. However, competition for resources may exist between AOA
536 abundance and FOL. This hypothesis was corroborated by the low nitrification rate that was
537 observed at the rhizosphere level especially in the untreated control. Our findings indicated that
538 nitrification may play an important role in disease control when environmental conditions for
539 nitrifiers are favourable. Similarly, Fujiwara et al., (2013) showed that, in a multiple parallel
540 mineralization system, rhizosphere microbiota suppressed the *Fusarium* wilt disease of lettuce by
541 affecting the morphological characteristics of *F. oxysporum*. However, further research is needed to
542 obtain a better understanding of the promoting effect of nitrifiers after the application of BCAs.

543

544 **Conclusions and outlook**

545 Our results have shown that the application of different biological control agents and compost
546 resulted in a significant reduction in the Fusarium wilt of lettuce crops, without any significant
547 differences in the degree of disease control among treatments. On the basis of the presented results,
548 it is possible to conclude that the applied treatments (based on *Bacillus*, *Pseudomonas* and
549 *Trichoderma*) did not affect negatively the density of resident microbial communities (i.e., bacteria
550 archaea and fungi) or the nitrifying prokaryotes. Nevertheless, the archaeal nitrifiers had a more
551 dynamic response after the treatments. Hence, more investigations are needed to study the influence
552 of BCA and compost treatments on archaeal communities. In addition, our results have shown that
553 lettuce plants could favour bacterial species which are good lettuce rhizosphere colonizers and may
554 act as antagonists.

555 Although no negative effects of BCAs were observed in this study on the no-target rhizosphere or
556 on the soil microbial biomass, their application requires further evaluations, including the
557 consideration of a broader range of soils with different physico-chemical properties, as well as
558 additional soil-borne pathogens and different plant species/cultivars, as they may have various
559 effects on the microbial communities and processes in the rhizosphere and bulk soil (Soderberg et
560 al., 2002; Rasche et al., 2006). In addition, the long-term effect on the same crop should be
561 evaluated in order to fully understand the legacy and the results of the interactions of BCAs and
562 compost with microbial biomass and activity. Although this study was focused on the potential
563 functionality of selected microbial populations in terms of abundance it can be assumed that the
564 introduction of different microbial strains or strain combinations could also influence the structure
565 and composition of the overall microbial communities. Therefore, advanced studies on the potential
566 effects of BCAs and organic matrices on the composition and biomass of non-target microbial
567 populations in the crop rhizosphere and bulk soil, using molecular fingerprinting techniques, are
568 still needed.

569

570 **Acknowledgements**

571 This project has received funding from the European Union's Horizon 2020 research and innovation
572 programme under grant agreement No. 633999 (EUCLID). The authors would kindly like to thank
573 Marguerite Jones for the language revision.

574

575 Conflict of interest

576 The authors declare that they have no conflict of interest.

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References

- Barrière, V., Lecompte, F., Nicot, P. C., Maisonneuve, B., Tchamitchian, M., Lescourret, F., 2014. Lettuce cropping with less pesticides. A review. *Agron. Sustain. Dev.* 34, 175- 198.
- Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. *ISME J.* 5, 908–917.
- Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Micro. Biotech.* 84, 11-18.
- Bergmark, L., Poulsen, P.H.B., Al-Soud, W.A., Norman, A., Hansen, L.H., Sørensen, S.J., 2012. Assessment of the specificity of *Burkholderia* and *Pseudomonas* qPCR assays for detection of these genera in soil using 454 pyrosequencing. *FEMS Microbiol. Lett.* 333, 77–84.
- Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Supression of soilborne fungal diseases with organic amendments. *Plant Pathol. J.* 89, 311-324.
- Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biol. Biochem.* 42, 136 - 144.
- Bonilla, N., Gutiérrez-Barranquero, J.A., de Vicente A., Cazorla F.M., 2012. Enhancing soil quality and plant health through suppressive organic amendments. *Diversity* 4, 475-491.
- Cao, Y., Zhang, Z.H., Ling, N., Yuan, Y.J., Zheng, X.Y., Shen, B. and Shen, Q.R., 2011. *Bacillus subtilis* SQR 9 can control Fusarium wilt in cucumber by colonizing plant roots. *Biol. Fert. Soil.* 47, 495–506.

621 Cawoy, H., Bettiol, W., Fickers, P., and Ongena, M., 2011. *Bacillus*-based biological control of
 622 plant diseases. In Pesticides in the modern world - Pesticides use and management, Stoytcheva, M.,
 623 (ed.), ISBN: 978-953-307-459-7.

624 Cucu, M.A., Marchan, S., Said-Pullicino, D., Celi, L., Kandeler, E., Rasche, F., 2017. Resource
 625 driven community dynamics of NH_4^+ assimilating and N_2O reducing archaea in a temperate paddy
 626 soil. *Pedobiologia* 62,16-27.

627 Dudenhofer, J.H., Scheu, S., Jousse, A., 2016. Systemic enrichment of antifungal traits in
 628 the rhizosphere microbiome after pathogen attack. *J Ecol.* 104, 1566–1575.

629 EUROSTAT, 2012, <http://ec.europa.eu/eurostat/web/lucas/data/primary-data/2012>.

630 FAO, 2014, Food and Agriculture Organization of the United Nations (FAO), 2014. Statistics
 631 Division, Production/Crops. <http://faostat3.fao.org/browse/Q/QC/E> (Accessed 07.28.2016).

632 Franceschini, S., Pugliese, M., Garibaldi, A., Gullino, M.L., 2016. Microbiological features
 633 associated with the suppression of fusarium wilt of lettuce studied by applying a single selected
 634 compost. *Compost Sci. Util.* 24 (3), 182–189.

635 Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and
 636 diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl.*
 637 *Acad. Sci. USA* 102, 14683–14688.

638 Fu, L., Penton, R., Ruan, Y., Shen, Z., Xue, C., Li, R., Shen, Q., 2017. Inducing the rhizosphere
 639 microbiome by biofertilizer application to suppress banana Fusarium wilt disease. *Soil Biol.*
 640 *Biochem.* 104, 39-48.

641 Fujiwara, K., Iida, Y., Iwai, T., Aoyama, C., Inukai, R., Ando, A., et al., 2013. The rhizosphere
 642 microbial community in a multiple parallel mineralization system suppresses the pathogenic fungus
 643 *Fusarium oxysporum*. *MicrobiologyOpen*, 2(6), 997–1009. <http://doi.org/10.1002/mbo3.140>.

644 Garibaldi, A., Gilardi, G., Gullino, M.L., 2002. First report of *Fusarium oxysporum* on lettuce in
 645 Europe. *Plant Dis.* 86, 1052.

646 Garibaldi, A., Gilardi, G., Gullino, M.L., 2004. Seed transmission of *Fusarium oxysporum* f. sp.
647 *lactucae*. Phytoparasitica 32, 61–65.

648 Gao, W., Zhang, W., Meldrum, D.R., 2011. RT-qPCR based quantitative analysis of gene
649 expression in single bacterial cells. J. Microbiol. Meth. 85, 221-227.

650 Gantner, S., Andersson, A. F., Alonso-Sáez, L., Bertilsson, S., 2011. Novel primers for 16S rRNA-
651 based archaeal community analyses in environmental samples. J. Microbiol. Methods 84, 12–18.

652 Ghini, R., Mezzalama, M., Ambrosoli, R., Barberis, E., Garibaldi, A., De Stefano Piedade, S.M.,
653 2000. *Fusarium oxysporum* strains as biocontrol agents against *Fusarium* wilt: effects on soil
654 microbial biomass and activity. Pesquisa Agropecuaria Brasileira 35, 93-101.

655 Gilardi, G., Garibaldi, A., Gullino, M.L., 2007. Effect of antagonistic *Fusarium* spp. and of
656 different commercial biofungicide formulations on *Fusarium* wilt of lettuce. Phytoparasitica 35,
657 457–465.

658 Gilardi, G., Pugliese, M., Gullino, M.L., Garibaldi, A., 2013. Problemi fitosanitari nella produzione
659 di insalate per la IV gamma. Giornata di Studio, Battipaglia (SA), 30 aprile 2013.
660 http://www.aipp.it/UserFiles/File/ProtezioneColtureIVGamma2013/02_-_Pugliese.pdf

661 Gilardi, G., Pugliese, M., Gullino, M.L., Garibaldi, A., 2016. Effect of different organic
662 amendments on lettuce *Fusarium* wilt and on selected soilborne microorganisms. Plant Pathol. 65,
663 704–712.

664 Gilardi G., Gullino M.L., Garibaldi A., 2017. Soil disinfestation with dimethyl disulfide for
665 management of *Fusarium* wilt on lettuce in Italy. J. Plant Dis. Prot. 124, 361–370.

666 Gordon, T.R., Koike, S.T., 2015. Management of *Fusarium* wilt of lettuce. Crop Prot. 73, 45-49.

667 Gullino, M.L., Migheli, Q., Mezzalama, M., 1995. Risk analysis for biological control agents:
668 antagonistic *Fusarium* spp. as a case study. Plant Disease 79, 1193-1201.

669 Gupta, R., Bru, D., Bisaria, V.S., Philippot, L., Sharma, S., 2012. Responses of *Cajanus cajan* and
670 rhizospheric N-cycling communities to bioinoculants. Plant Soil 358, 143–154.

671 Gyaneshwar, P., Kumar, G.N., Parekh, L.J., and Poole, P.S., 2002. Role of soil microorganisms in
672 improving P nutrition of plants. *Plant Soil* 245, 83-93.

673 Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent
674 pseudomonads. *Nat. Rev. Microbiol.* 3(4), 307-19.

675 Hagn, A., Wallisch, S., Radl, V., Munch, J.C., Schloter, M., 2007. A new cultivation independent
676 approach to detect and monitor common *Trichoderma* species in soils. *J. Microbiol. Methods.* 69,
677 86–92.

678 Hoffmann, H., Schloter, M., Wilke, B.-M., 2007. Micro-scale measurement of potential nitrification
679 rates of soil aggregates. *Biol Fertil Soil* 44, 411 – 413.

680 Innocenti, G., Roberti, R., Piattoni, F., 2015. Biocontrol ability of *Trichoderma harzianum* strain
681 T22 against Fusarium wilt disease on water-stressed lettuce plants. *BioControl*, 60, 573-581.

682 Karlsson, A. E., Johansson, T. and Bengtson, P., 2012. Archaeal abundance in relation to root and
683 fungal exudation rates. *FEMS Microbiol. Ecol.*, 80, 305–311.

684 Kloepper, J.W., Ryu, C.M., and Zhang, S., 2004. Induced systemic resistance and promotion of
685 plant growth by *Bacillus* spp. *Symposium.* 94, 1259-1266.

686 Lane, D., 1991. 16S/23S rRNA sequencing. In: Stackebrandt A, Goodfello M (eds) *Nucleic acid*
687 *techniques systematics*, 1st edn. Wiley, West Sussex, pp. 115–257.

688 Larkin, R.P., 2015. Soil health paradigms and implications for disease management. *Annu. Rev.*
689 *Phytopathol.* 53, 199–221.

690 Ling, N., Xue, C., Huang, Q., Yang, X., Xu, Y., Shen, Q., 2010. Development of a mode of
691 application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt.
692 *BioControl.* 55 (5), 673–683.

693 Loc, N.H., Quang, H.T., Hung, N.B., Huy, N.D., Phuong, T.T., Ha, T.T., 2011. *Trichoderma*
694 *asperellum* Chi42 genes encode chitinase. *Mycobiol.* 39(3), 182-186.

695 Loper, J.E., Hassan, K.A., Mavrodi, D.V., Davis, E.W. II, Lim, C.K., et al., 2012. Comparative
696 genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits

involved in multitrophic interactions. PLOS Genetics 8(7), e1002784.
<https://doi.org/10.1371/journal.pgen.1002784>.

Luo, J., Ran, W., Hu, J., Yang, X.M., Xu, Y.C., Shen, Q.R., 2009. Application of bio-organic fertilizer significantly affected fungal diversity of soils. Soil Sci. Soc. Am. J. 74, 2039–48.

McSpadden Gardener, B.B., Mavrodi, D.V., Thomashow, L.S., Weller, D.M., 2001. A rapid polymerase chain reaction-based assay characterizing rhizosphere populations of 2,4-diacetylphloroglucinol-producing bacteria. Phytopathol. 91, 44–54.

Matheron, M.E., Porchas, M., 2010. Evaluation of soil solarization and flooding as management tools for Fusarium wilt of lettuce. Plant Dis. 94, 1323-1328.

Matheron, M.E., Gullino, M.L., 2012. Fusarium wilts of lettuce and other salad crops. In: Gullino, M.L., Katan, J., Garibaldi, A. (eds.). Fusarium wilts of greenhouse vegetable and ornamental crops. APS Press, St.Paul, MN. pp. 175-183.

Mendez, R., Garbeva, P., Raaijmakers, L.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37 (5), 634-663.

Mezzalana, M., Ghini, R., Ruffa, P., Ambrosoli, R., Garibaldi, A., 1998. Effect of antagonistic *Fusarium oxysporum* on functional groups of indigenous bacteria in soil. Appl. Soil Ecol. 7, 31-40.

Musyoki, M.K., Cadish, G., Enowashu, E., Zimmermann, J., Muema E., Beed, F., Rasche, F., 2015. Promoting effect of *Fusarium oxysporum* [f.sp. *strigae*] on abundance of nitrifying prokaryotes in a maize rhizosphere across soil types. Biol. Control. 83, 37-45.

Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.

Nel, B., Steinberg, C., Labuschagne, N., Viljoen A., 2006. The potential of nonpathogenic *Fusarium oxysporum* and other biological control organisms for suppressing Fusarium wilt of banana. Plant Pathol. 55, 217–223.

723 Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the
 724 diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria.
 725 Environ. Microbiol. 11, 2966–2978.

726 Olsen, S., Cole, C., Watanabe, F., Dean, L., 1954. Estimation of available phosphorus in soils by
 727 extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington,
 728 D.C.

729 Orr R., and Nelson P.N., 2018. Impacts of soil abiotic attributes on Fusarium wilt, focusing on
 730 bananas. Appl. Soil. Ecol. <https://doi.org/10.1016/j.apsoil.2018.06.019>.

731 Pane, C., Piccolo, A., Spaccini, R., Celano, G., Villeco, D., Zaccardelli, M., 2013. Agricultural
 732 waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-
 733 borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. Appl. Soil. Ecol. 65, 43-51.

734 Pugliese, M., Liu, B.P., Gullino, M.L., Garibaldi A., 2008. Selection of antagonists from compost to
 735 control soil-borne pathogens. J. Plant Dis. Protect. 115, 220-228.

736 Pugliese, M., Liu, B.P., Gullino, M.L., Garibaldi, A., 2011. Microbial enrichment of compost with
 737 biological control agents to enhance suppressiveness to four soil-borne diseases in green house. J.
 738 Plant Dis. Protect. 118, 45-50.

739 Pugliese, M., Gilardi, G., Garibaldi, A., Gullino, M.L., 2015. Organic amendments and soil
 740 suppressiveness: results with vegetable and ornamental crops. Soil. Biol. 46, 495-509.

741 Qiu, M., Zhang, R., Xue C., Zhang S., Zhang S.L., Shen, Q., 2012. Application of bio-organic
 742 fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of
 743 rhizosphere soil. Biol. Fert. Soils 48, 807-816.

744 Rasche, F., Hödl, V., Poll, C., Kandeler, E., Gerzabek, M.H., van Elsas, J.D., Sessitsch, A. 2006.
 745 Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the
 746 effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiol. Ecol.
 747 56 (2), 219-235.

748 Richardson, A.E., Barea, J., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus
 749 and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*, 339,
 750 305–339.

751 Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene
 752 amoA as a functional marker: molecular fine-scale analysis of natural ammonia oxidizing
 753 populations. *Appl. Environ. Microbiol.* 63, 4704–4712.

754 Shen, Z., Ruan, Y., Xue, C., Zhong, S., Li, R., Shen Q., 2015. Soils naturally suppressive to banana
 755 *Fusarium* wilt disease harbor unique bacterial communities. *Plant Soil*. 1-2, 21-33.

756 Shimazu, J., Yamauchi, N., Hibi, T., Satou, M., Horiuchi, S., Shirakawa, T., 2005. Development of
 757 sequence tagged site markers to identify races of *Fusarium oxysporum* f.sp. *lactucae*. *J. Gen. Plant*
 758 *Pathol.* 71, 183-189.

759 Söderberg, K.H., Olsson, P.A., Baath, E., 2002. Structure and activity of the bacterial community in
 760 the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonisation.
 761 *FEMS Microbiol. Ecol.* 40, 223–231.

762 Srinivasan, K., Gilardi, G., Garibaldi, A., Gullino, M.L., 2009. Bacterial antagonists from used
 763 rockwool soilless substrates suppress *Fusarium* wilt of tomato. *J Plant Pathol.* 91, 145-152.

764 Strange, R. N., and Scott, P. R., 2005. Plant disease: a threat to global food security. *Annu. Rev.*
 765 *Phytopathol.* 43, 83–116.

766 Svercel, M., Duffy, B., Défago, G., 2007. PCR amplification of hydrogencyanide biosynthetic locus
 767 *hcnAB* in *Pseudomonas* spp. *J. Microbiol. Meth.* 70, 209–213.

768 Vainio, E.J., Hantula, J., 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient
 769 gel electrophoresis of amplified ribosomal DNA. *Mycol. Res.* 104, 927—936.

770 Valentine, D.L., 2007. Adaptations to energy stress dictate the ecology and evolution of the
 771 Archaea. *Nature Rev. Microbiol.* 5, 316-323.

772 Weller, D. M., Raaijmakers, J. M., McSpadden Gardener, B. B., and Thomashow, L. S. 2002.
773 Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev.*
774 *Phytopathol.* 40:309-348.

775 Weller, D.M., 2007. *Pseudomonas* biocontrol agents of soilborne pathogen: looking back over 30
776 years. *Phytopathology.* 97, 250–256.

777 Wessén, E., Nyberg, K., Jansson, J.K., Hallin, S., 2010. Responses of bacterial and archaeal
778 ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Appl.*
779 *Soil Ecol.* 45, 193-200.

780 Wu, H.S., Yang, X.N., Fan, J.Q., Miao, W.G., Ling, N., Xu, Y.C., Huang, Q.W., Shen, Q.R., 2009.
781 Suppression of *Fusarium* wilt of watermelon by a bio-organic fertilizer containing combinations of
782 antagonistic microorganisms. *BioControl.* 54, 287-300.

783 Yang, X.M., Chen, L.H., Yong, X.Y., Shen, Q.R., 2011. Formulations can affect colonization and
784 biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against *Fusarium* wilt of cucumbers.
785 *Biol. Fertil. Soils.* 47, 239–248.

786 Zhang, S., Raza W., Yang, X., Hu, J., 2008. Control of *Fusarium* wilt disease of cucumber plants
787 with the application of a bioorganic fertilizer. *Biol. Fertil. Soils.* 44, 1073-1080.

788 Zhang, X., Liu, W., Schlöter, M., Zhang, G., Chen, Q., et al., 2013. Response of the abundance of
789 key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS ONE.* 8(10),
790 e76500. doi:10.1371/journal.pone.0076500.

791 Zimmermann, J., Musyoki, M.K., Cadisch, G., Rasche, F., 2016. Proliferation of the biocontrol
792 agent *Fusarium oxysporum* f. sp. *strigae* and its impact on indigenous rhizosphere fungal
793 communities in maize under different agro-ecologies. *Rhizosphere.* 1, 17-25.

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798 **Figure Captions**

799 Figure 1 Abundance of bacterial (a), archaeal (b) 16S rRNA genes and fungal (c) 18S rRNA genes
800 in rhizosphere and bulk soil between different treatments* and the untreated control (SM - Serenade
801 Max; RM - Remedier; Pp - *Pseudomonas*; CM – Green compost plus *Trichoderma* TW2; CV -
802 green compost; TW2 - *Trichoderma* TW2; C - control) (n = 6, means±standard errors). Different
803 letters above bars indicate significant differences between treatments in rhizosphere (uppercase
804 letters) and bulk soil (lowercase letters)

807 Figure 2 Potential Nitrification Activity (PNA) in the rhizosphere and bulk soil after different
808 treatments* and the untreated control (SM - Serenade Max; RM - Remedier; Pp - *Pseudomonas*;
809 CM - Green compost plus *Trichoderma* TW2; CV - green compost; TW2 - *Trichoderma* TW2; C -
810 control) (n = 6, means±with standard errors). Different letters above bars indicate significant
811 differences between treatments in rhizosphere (uppercase letters) and bulk soil (lowercase letters)

813 *Serenade max – SM: *Bacillus subtilis* ; Remedier – RM : *Trichoderma asperellum* + *T. gamsii*; *Pseudomonas putida* – Pp; ANT’S COMPOST M –
814 CM: Green compost + *Trichoderma* TW2; ANT’S COMPOST V – CV: Green compost; *Trichoderma* sp. – TW2

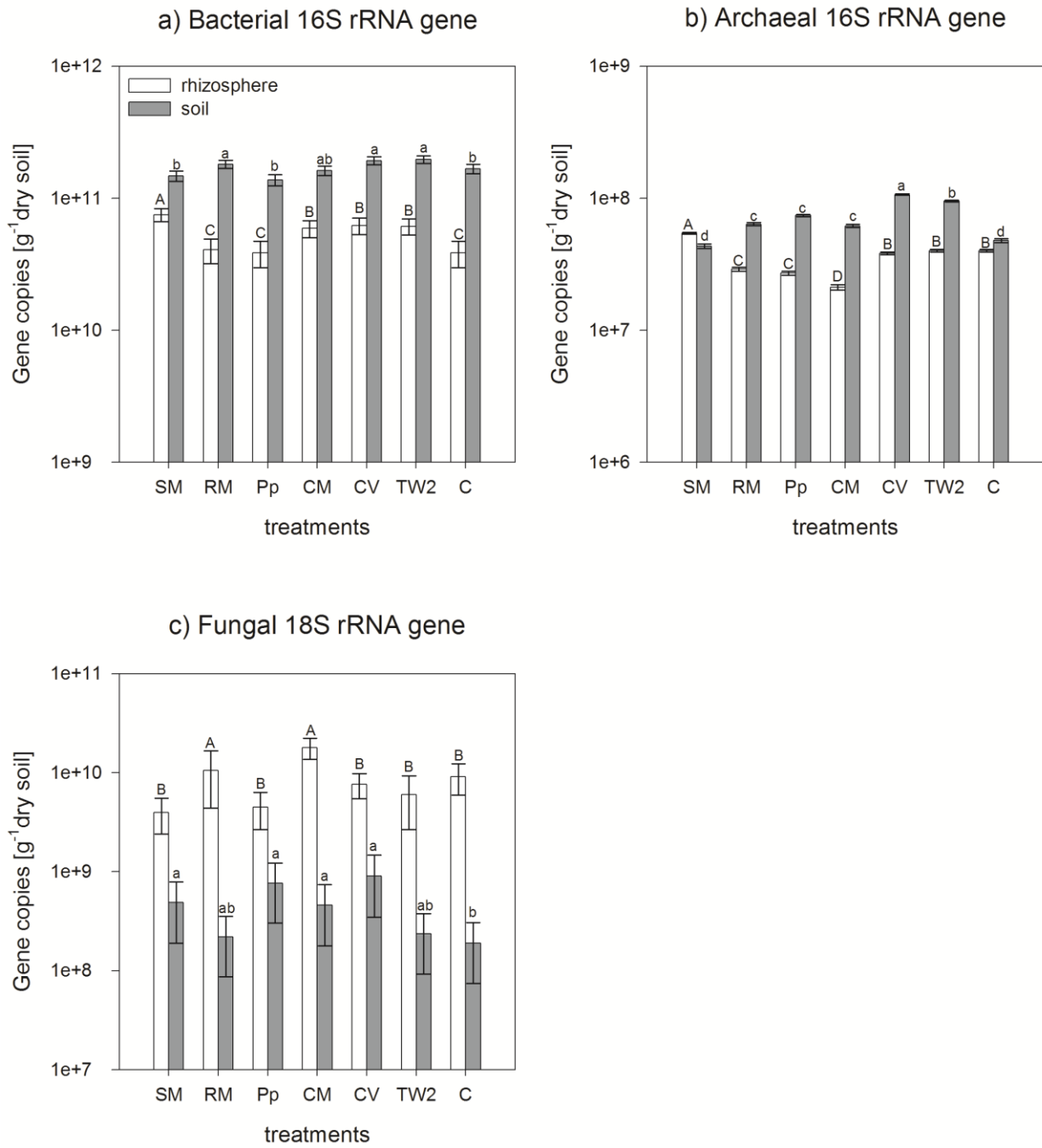
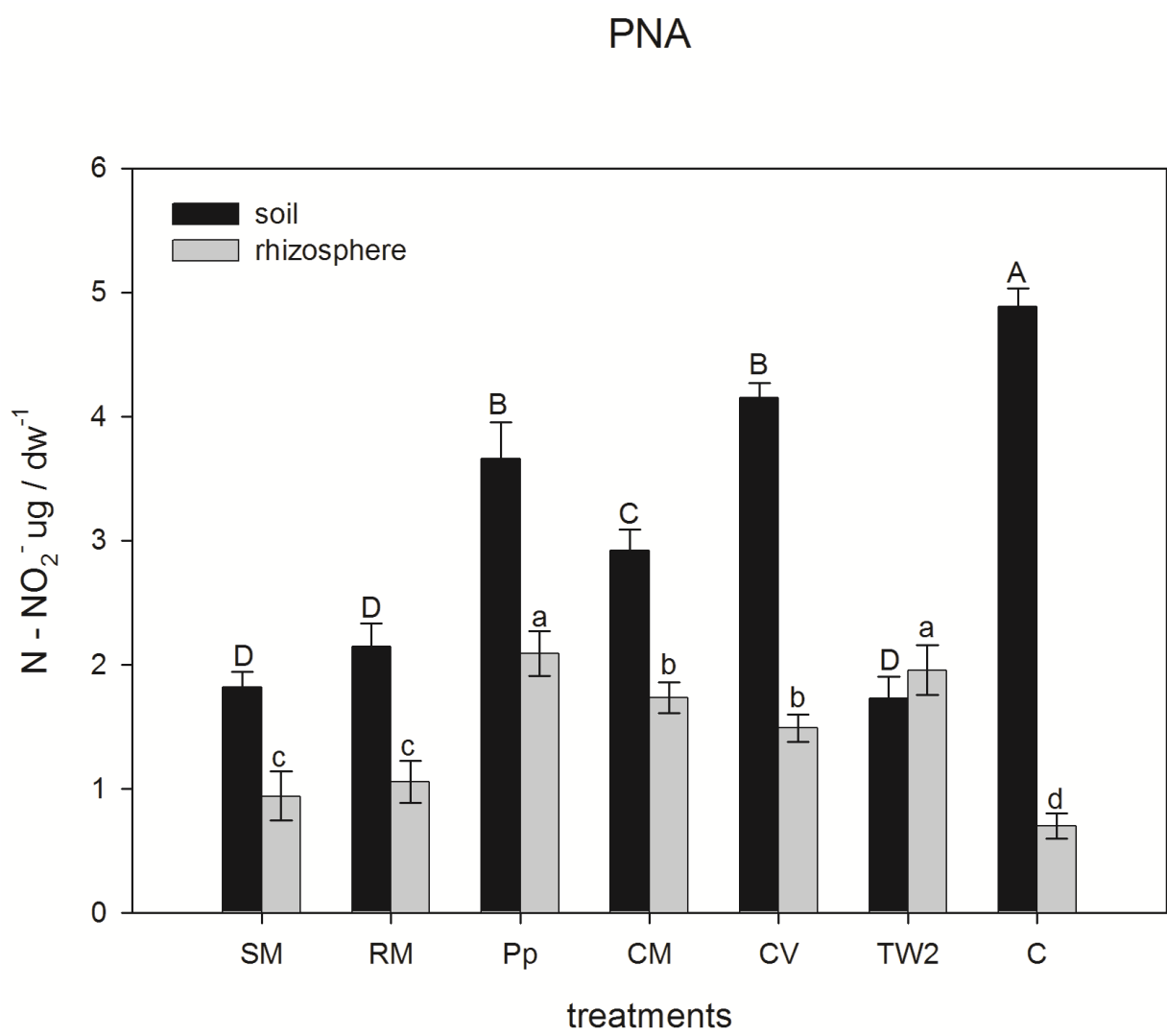


Figure1

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846 Figure2

Supplementary material

Table S1 Chemical properties of the rhizosphere (rhizo) and bulk soil for all the treatments and the untreated control at the end of trial 1 (2016) and trial 2 (2017). Values are given as average (n = 3). Different letters within a row show significant differences between treatments and the untreated control.

Property	Serenade Max		Remedier		Pseudomonas		Compost M		Compost V		Trichoderma		Control	
	Rhizo	Bulk soil	Rhizo	Bulk soil	Rhizo	Bulk soil	Rhizo	Bulk soil	Rhizo	Bulk soil	Rhizo	Bulk soil	Rhizo	Bulk soil
2016														
pH	5.76±0.1 ^d	6.34±0.1 ^d	6.01±0.2 ^b	6.67±0.2 ^b	5.98±0.2 ^c	6.73±0.1 ^a	6.23±0.1 ^a	6.61±0.1 ^b	5.92±0.2 ^c	6.59±0.2 ^b	6.03±0.2 ^b	6.6±0.1 ^b	5.80±0.1 ^d	6.53±0.1 ^b
TN %	0.57±0.2 ^d	0.21±0.05 ^a	0.50±0.2 ^d	0.19±0.08 ^a	0.27±0.3 ^f	0.21±0.04 ^a	0.34±0.1 ^e	0.21±0.05 ^a	0.83±0.1 ^b	0.20±0.03 ^a	0.62±0.3 ^c	0.20±0.08 ^a	1.02±0.1 ^a	0.20±0.0 ^a
NH ₄ ⁺ (mg/kg)	2.9±0.9 ^d	0.23±0.09 ^b	1.6±0.6 ^e	0.39±0.2 ^a	2.07±0.8 ^c	0.27±0.1 ^b	1.13±0.5 ^f	0.29±0.1 ^b	5.29±0.1 ^b	0.33±0.1 ^b	0.6±0.1 ^g	0.36±0.1 ^a	37.55±1.7 ^a	0.42±0.1 ^a
NO ₃ ⁻ (mg/kg)	18.06±1.2 ^g	5.91±1.9 ^f	22.65±1.8 ^e	8.51±1.6 ^e	39.28±1.9 ^b	22.86±1.7 ^a	30.0±0.1 ^d	16.83±0.1 ^c	36.0±0.1 ^c	14.30±0.1 ^d	19.15±0.1 ^f	20.58±0.1 ^b	46.43±0.1 ^a	8.62±0.1 ^a
TOC %	19.89±0.1 ^c	1.87±0.1 ^a	13.82±0.1 ^d	1.90±0.1 ^a	20.51±0.1 ^c	1.90±0.1 ^a	7.21±0.1 ^e	1.96±0.1 ^a	31.22±0.1 ^b	1.90±0.1 ^a	19.34±0.1 ^c	1.89±0.1 ^a	43.36±0.1 ^a	1.71±0.1 ^a
TP (mg/kg)	906±1.5 ^f	1262.4±2.1 ^b	1020±1.2 ^d	1337.3±0.1 ⁵ ^a	1296±1.2 ^b	1339.7±1.2 ^a	1401±1.6 ^a	1264.9±2.1 ^b	949±1.5 ^e	1254±2.1 ^b	1191±2.2 ^c	1238±2.5 ^b	679±1.5 ^g	1237±2.5 ^a
AP (mg/kg)	124.4±0.2 ^b	150.2±2.7 ^c	118.5±0.5 ^c	152.7±2.3 ^b	129.3±0.7 ^a	156.2±2.1 ^a	125.9±0.1 ^b	158±2.31 ^a	93.3±0.2 ^d	159.7±1.8 ^a	113.5±0.1 ^c	156.6±2.6 ^a	83.5±0.1 ^e	159.5±2.1 ^a
2017														
pH	5.70±0.1 ^b	6.56±0.1 ^c	6.1±0.23 ^a	6.88±0.1 ^a	5.70±0.2 ^b	6.77±0.1 ^b	6.43±0.1 ^a	6.76±0.1 ^b	5.85±0.1 ^b	6.74±0.2 ^b	5.98±0.2 ^b	6.9±0.2 ^a	5.7±0.1 ^b	6.55±0.1 ^a
TN %	0.67±0.1 ^d	0.12±0.07 ^c	0.47±0.1 ^e	0.21±0.05 ^b	0.33±0.2 ^f	0.32±0.04 ^a	0.35±0.1 ^f	0.19±0.05 ^b	0.97±0.2 ^b	0.12±0.05 ^c	0.79±0.3 ^c	0.18±0.05 ^b	1.20±0.2 ^a	0.18±0.0 ^a
NH ₄ ⁺ (mg/kg)	2.59±0.5 ^c	0.25±0.1 ^d	2.6±0.17 ^e	0.41±0.1 ^b	2.9±0.5 ^d	0.25±0.2 ^c	2.27±0.2 ^f	0.3±0.1 ^c	4.43±0.4 ^b	0.27±0.2 ^b	0.5±0.2 ^g	0.41±0.1 ^b	33.76±1.2 ^a	0.56±0.1 ^a
NO ₃ ⁻ (mg/kg)	18.31±1.2 ^e	6.35±1.5 ^f	25.83±1.2 ^d	7.67±1.5 ^e	45.28±1.6 ^a	25.55±1.5 ^a	36.0±1.2 ^c	19.91±1.2 ^c	39.0±1.3 ^b	12.47±1.2 ^d	19.21±1.2 ^e	22.47±1.4 ^b	51.29±1.2 ^c	5.37±1.2 ^a
TOC %	21.35±2.1 ^d	2.56±1.2 ^b	15.79±2.3 ^e	3.2±1.2 ^a	22.34±2.1 ^d	2.2±1.2 ^c	13.33±2.2 ^f	2.85±2.4 ^a	27.46±2.2 ^c	2.3±2.3 ^c	32.34±2.3 ^b	1.89±1.2 ^d	56.36±1.3 ^a	1.71±2.1 ^a
TP (mg/kg)	1023±1.5 ^d	1357±2.1 ^a	980±1.2 ^d	975±2.1 ^d	1136±1.4 ^c	1238±1.2 ^b	1521±2.1 ^a	1132.9±1.5 ^c	1178±2.1 ^c	1376±1.2 ^a	1275±2.1 ^b	1231±1.2 ^b	53±2.1 ^e	1129±1.6 ^a
AP (mg/kg)	112.9±0.1 ^c	176.1±2.1 ^b	131.4±0.5 ^b	164.6±2.2 ^b	131.7±0.5 ^a	175.4±2.1 ^b	131.2±0.1 ^b	187±2.1 ^a	112±0.2 ^c	198.5±2.1 ^a	99.5±0.2 ^c	153±2.1 ^c	76±0.1 ^d	171±2.2 ^a

Table S2 - Linear regressions (R² correlation coefficients, n=21) between microbial abundance and chemical properties in rhizosphere (rhizo) and bulk soil samples

Property	16S bacteria		16S archaea		18S fungi		<i>chiA gene</i>		AOA		AOB	
	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil
pH	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TN	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.38*	0.673*	0.77***	<i>ns</i>	0.876	<i>ns</i>
NH ₄ ⁺	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.754***	0.85***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
NO ₃ ⁻	0.656***	<i>ns</i>	0.778***	0.697**	<i>ns</i>	<i>ns</i>	0.68***	0.50**	0.79***	0.777***	0.65**	0.856***
Ptot	0.50**	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.679***	0.95***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Pav	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TOC	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	6.975***	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.76***	<i>ns</i>	<i>ns</i>	<i>ns</i>

Significance levels: not significant-ns: p>0.05; *p<0.05; **p<0.01; ***p<0.001.

883 Tables

884 Table 1 – General information on the trials and timing of the operations carried out in 2016 (trial 1)
885 and 2017 (trial 2)

Treatment	Microorganism - active ingredient	Dosage	Tray treatment	Plot Treatment
Serenade max - SM	<i>Bacillus subtilis</i> QST 713	2.9X10 ¹⁰ cells/L water	T5; T10; T15;T20*	-
Remedier - RM	<i>Trichoderma asperellum</i> + <i>T gamsii</i>	1.2X10 ⁶ cells/L water	T5; T10; T15;T20	-
<i>Pseudomonas putida</i> - Pp	FC7B+ FC8B +FC9B	1x10 ⁷ (cells /ml water)	T5; T10; T15;T20	-
ANT'S COMPOST M - CM	Green compost + <i>Trichoderma</i> TW2	8g/seedling;1kg/0.2m ³ of soil	T0	- T20
ANT'S COMPOST V - CV	Green compost	8 g/seedling; 1kg/0.1m ³ of soil	T0	- T20
<i>Trichoderma</i> sp. - TW2	<i>Trichoderma</i> strain TW2	1x10 ⁷ (cells /ml)	T5; T10; T15; T20	
Ortiva	Azoxystrobin	0.19 g a.i./Lwater		T20
Untreated control - C	-	-		

886 *Treatments: T0 at sowing; T5: 5 days after sowing; T10: 10 days after sowing; T15: 15 days
887 after sowing; T 20: 20 days after sowing and immediately before transplanting.

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891 Table 2 Description of the primer sets and amplification details used for the quantitative PCR.

Target group	Primer (reference)	Amplification details
<i>Fusarium</i>	FLA0001F (Shimazu et al., 2005)	40 cycles
<i>oxysporum</i> f.sp. <i>lactucae</i> (FOL)	FLA0001R (Shimazu et al., 2005)	95°C 15s, 60°C 60s, 72°C 45s
All bacteria	Eub338 (Lane 1991)	40 cycles
(16S rRNA gene)	Eub518 (Muyzer et al. 1993)	95°C 30s, 55°C 35s, 72°C 45s
All archaea	340F (Gantner et al., 2011)	40 cycles
(16S rRNA gene)	1000R (Gantner et al., 2011)	95°C 30s, 57°C 30s, 72°C 30s
All fungi	FR1 (Vainio and Hantula, 2000)	45 cycles
(18S rRNA gene)	390FF (Vainio and Hantula, 2000)	95°C 30s, 50°C 30s, 70°C 60s
Ammonia oxidizing bacteria (AOB)	AmoA-1f (Rotthauwe et al. 1997)	45 cycles
	AmoA-2r (Rotthauwe et al. 1997)	95°C 30s, 57°C 45s, 72°C 45s, 78°C 20s
Ammonia oxidizing archaea (AOA)	Arch-amoAf (Francis et al., 2005)	45cycles
	Arch-amoAr (Francis et al.,2005)	95°C 30s, 53°C 45s, 72°C 45s, 78°C 20s
<i>chiA</i> gene	chiaxf (this study)	35cycles
	chiaxr (this study)	95°C 15s, 59.9°C 30s, 70°C 30s
<i>phlD</i> gene	BPF2 (McSpadden Gardener et al., 2001)	40 cycles
	BPR4 (McSpadden Gardener et al., 2001)	95°C 15s, 60°C 45s, 72°C 45s, 78°C 20s
<i>hcnAB</i> gene	PM2 (Svercel, Duffy & Défago 2007)	40 cycles
	PM-26R (Svercel, Duffy & Défago 2007)	95°C 15s, 60°C 45s, 72°C 45s, 78°C 20s
<i>Bacillus</i>	Forward <i>B. subtilis</i> (Gao et al., 2011)	40 cycles
	Reverse <i>B. subtilis</i> (Gao et al., 2011)	95°C 30s, 60°C 60s, 80°C 10s
<i>Trichoderma</i>	uTf (Hagn et al., 2007)	35 cycles
	uTr (Hagn et al., 2007)	95 °C 30 s, 55.5 °C 30 s, 72 °C 30 s
<i>Pseudomonas</i>	Pse435F (Bergmark et al., 2012)	40 cycles
	Pse686R (Bergmark et al., 2012)	95°C 30s, 60°C 60s, 80°C 10s

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896 Table 3 Effect of the preventative soil treatments with BCAs and compost on disease severity and fresh weight at the end of trials 1 and 2, 2016 and
897 2017, respectively

Treatment	Microorganism - active ingredient	Disease severity*				Fresh weight g/12 plants			
		2016		2017		2016		2017	
Serenade max - SM	<i>Bacillus subtilis</i> QST 713	26.9	±3.6 ^a	22.1	±3.3 ^{ab}	3135.6	±128.9 ^a	2727.2	±371.5 ^{ab}
Remedier - RM	<i>Trichoderma asperellum</i> + <i>T. gamsii</i>	27.7	±3.2 ^a	29.5	±7.0 ^{ab}	2731.0	±79.0 ^a	2868.4	±348.7 ^{ab}
<i>Pseudomonas putida</i> - Pp	FC7B+ FC8B +FC9B	28.1	±2.9 ^a	35.2	±6.3 ^{ab}	3090.7	±49.6 ^a	1528.0	±67.2 ^{bc}
ANT'S COMPOST M - CM	Green compost + <i>Trichoderma</i> TW2	25.3	±3.3 ^a	13.0	±4.7 ^a	3256.3	±267.6 ^a	3766.0	±481.6 ^a
ANT'S COMPOST V - CV	Green compost	32.0	±4.1 ^a	29.0	±5.3 ^{ab}	3323.9	±146.7 ^a	3747.6	±130.1 ^a
<i>Trichoderma</i> sp. - TW2	<i>Trichoderma</i> strain TW2	32.2	±2.7 ^a	36.4	±3.3 ^b	2815.7	±78.6 ^a	1917.6	±134.7 ^{bc}
Ortiva	Azoxystrobin	24.8	±4.0 ^a	19.8	±2.3 ^{ab}	3014.0	±185.3 ^a	3360.4	±273.0 ^a
Untreated control - C	-	61.0	±3.5 ^b	61.7	±4.1 ^c	1745.7	±93.0 ^b	770.8	±243.1 ^c

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900 *Sixteen plants/treatment were evaluated for disease severity using the rating scale: 0 = healthy plant, 25 = initial leaf chlorosis, 50 = severe leaf chlorosis and initial symptoms of
901 wilting during the hottest hours of the day, 75 = severe wilting and severe symptoms of leaf chlorosis; 100 = plant totally wilted, leaves completely necrotic.

902 Table 4 Abundance of *F. oxysporum* f.sp. *lactucae* (FOL) in the rhizosphere and bulk soil after
 903 different treatments* and in the untreated control at the end of trial 1 (2016) and trial 2 (2017)
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Treatment	Rhizosphere 2016		Bulk soil 2016		Rhizosphere 2017		Bulk soil 2017	
	<i>FOL (logcopy DNA⁻¹)</i>							
Serenade Max - SM	6.359	a	6.711	c	6.345	b	6.796	b
Remedier - RM	5.626	c	6.758	c	5.692	c	6.828	b
<i>P. putida</i> - Pp	6.034	b	7.117	b	6.179	b	6.959	a
ANT'S COMPOST M - CM	5.423	d	6.086	d	5.243	d	6.119	c
ANT'S COMPOST V - CV	5.897	c	6.769	c	5.897	c	6.821	b
<i>Trichoderma</i> sp. - TW2	6.122	b	5.950	d	6.292	b	5.983	c
Untreated control - C	6.445	a	7.298	a	7.635	a	7.073	a
<i>P(F) Treat</i>	0.000							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	ns							
<i>P(F) Rhizo*Bulk</i>	0.001							

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906 Values represent marginal means

907 *Serenade max – SM: *Bacillus subtilis* ; Remedier – RM : *Trichoderma asperellum* + *T. gamsii*; *Pseudomonas putida* – Pp; ANT’S COMPOST M –

908 CM: Green compost + *Trichoderma* TW2; ANT’S COMPOST V – CV: Green compost; *Trichoderma* sp. – TW2

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921 Table 5 Abundance of bacterial (AOB) and archaeal (AOA) *amoA* genes in the rhizosphere and
 922 bulk soil after different treatments* and in the untreated control at the end of trial 1 (2016) and trial
 923 2 (2017)

Treatment	Rhizosphere 2016		Bulk soil 2016		Rhizosphere 2017		Bulk soil 2017	
<i>AOB (logcopy DNA⁻¹)</i>								
Serenade Max - SM	7.417	a	7.374	c	7.493	a	7.383	c
Remedier - RM	7.139	c	7.653	b	7.192	c	7.741	b
<i>P. putida</i> - Pp	7.045	c	7.628	b	7.086	c	7.660	b
ANT'S COMPOST M - CM	7.236	b	7.680	b	7.320	b	7.715	b
ANT'S COMPOST V - CV	7.407	a	7.656	b	7.446	a	7.693	b
<i>Trichoderma</i> sp. - TW2	7.396	a	7.703	b	7.410	a	7.698	b
Untreated control - C	7.039	c	7.951	a	7.341	b	7.854	a
<i>P(F) Treat</i>	0.000							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	ns							
<i>P(F) Rhizo*Bulk</i>	0.007							
<i>AOA (logcopy DNA⁻¹)</i>								
Serenade Max - SM	7.057	a	8.829	c	7.100	a	8.834	b
Remedier - RM	6.653	b	8.744	c	6.826	b	8.830	b
<i>P. putida</i> - Pp	6.415	c	8.958	b	6.653	c	8.985	b
ANT'S COMPOST M - CM	6.613	b	8.823	c	6.690	c	8.828	b
ANT'S COMPOST V - CV	6.721	b	9.163	a	6.800	b	9.224	a
<i>Trichoderma</i> sp. - TW2	6.974	a	9.042	b	6.844	b	9.123	a
Untreated control - C	5.799	d	8.118	d	5.839	d	8.422	c
<i>P(F) Treat</i>	0.000							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	0.015							
<i>P(F) Rhizo*Bulk</i>	0.006							

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926 Values represent marginal means

927 *Serenade max – SM: *Bacillus subtilis* ; Remedier – RM : *Trichoderma asperellum* + *T. gamsii*; *Pseudomonas putida* – Pp; ANT’S COMPOST M –

928 CM: Green compost + *Trichoderma* TW2; ANT’S COMPOST V – CV: Green compost; *Trichoderma* sp. – TW2

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934 Table 6 Abundance of *chiaA*, *phlD*, and *hcnAB* genes in the rhizosphere and bulk soil after different
 935 treatments* and the untreated control at the end of trial 1 (2016) and trial 2 (2017)

Treatment	Rhizosphere 2016		Bulk soil 2016		Rhizosphere 2017		Bulk soil 2017	
<i>chiA</i> gene (logcopy DNA ⁻¹)								
Serenade Max - SM	4.528	c	4.406	b	4.572	d	4.539	b
Remedier - RM	4.761	ab	4.632	a	4.815	c	4.513	b
<i>P. putida</i> - Pp	4.317	d	4.612	a	4.482	d	4.635	a
ANT'S COMPOST M - CM	4.905	b	4.337	b	5.080	b	4.358	c
ANT'S COMPOST V - CV	4.292	d	4.566	a	4.334	e	4.621	a
<i>Trichoderma</i> sp. - TW2	5.676	a	4.433	b	5.711	a	4.520	b
Untreated control - C	4.327	d	4.385	b	3.915	f	4.284	d
<i>P(F) Treat</i>	0.000							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	ns							
<i>P(F) Rhizo*Bulk</i>	ns							
<i>phlD</i> gene (logcopy DNA ⁻¹)								
Serenade Max - SM	4.952	c	2.627	a	4.842	c	2.613	b
Remedier - RM	4.864	c	2.662	a	4.726	c	2.642	b
<i>P. putida</i> - Pp	5.371	a	2.166	b	5.554	a	2.198	c
ANT'S COMPOST M - CM	4.814	c	2.756	a	4.852	c	2.663	b
ANT'S COMPOST V - CV	5.119	a	2.280	b	5.159	b	2.249	c
<i>Trichoderma</i> sp. - TW2	5.248	b	2.957	a	5.260	b	2.942	a
Untreated control - C	4.950	c	2.786	a	3.959	d	2.621	b
<i>P(F) Treat</i>	ns							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	ns							
<i>P(F) Rhizo*Bulk</i>	0.013							
<i>hcnAB</i> gene (logcopy DNA ⁻¹)								
Serenade Max - SM	6.319	b	2.613	a	6.482	b	2.556	b
Remedier - RM	3.734	d	2.642	a	3.754	e	2.670	b
<i>P. putida</i> - Pp	6.707	a	2.198	b	6.555	b	2.155	c
ANT'S COMPOST M - CM	6.910	a	2.663	a	6.940	a	2.643	b
ANT'S COMPOST V - CV	5.601	d	2.249	b	5.636	c	2.227	c
<i>Trichoderma</i> sp. - TW2	6.810	a	2.942	a	6.391	b	2.892	a
Untreated control - C	4.950	c	2.791	a	4.864	d	2.811	a
<i>P(F) Treat</i>	ns							
<i>P (F) Year</i>	0.000							
<i>P(F) Treat*Year</i>	ns							
<i>P(F) Rhizo*Bulk</i>	0.016							

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937

938 Values represent marginal means

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940 *Serenade max – SM: *Bacillus subtilis* ; Remedier – RM : *Trichoderma asperellum* + *T. gamsii*; *Pseudomonas putida* – Pp; ANT’S COMPOST M –

941 CM: Green compost + *Trichoderma* TW2; ANT’S COMPOST V – CV: Green compost; *Trichoderma* sp. – TW2

942 Table 7 Abundance of *Bacillus*, *Pseudomonas*, and *Trichoderma* genes in the rhizosphere and bulk
 943 soil after different treatments* and the untreated control at the end of trial 1 (2016) and trial 2
 944 (2017)
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Treatment	Rhizosphere 2016		Bulk soil 2016		Rhizosphere 2017		Bulk soil 2017	
<i>Bacillus (logcopy DNA⁻¹)</i>								
Serenade Max - SM	5.039	a	5.394	a	5.087	a	5.477	a
Remedier - RM	4.249	c	4.109	d	4.194	b	4.130	d
<i>P. putida</i> - Pp	3.989	c	4.320	c	3.980	c	4.331	c
ANT'S COMPOST M - CM	4.702	b	4.790	b	4.308	b	4.814	b
ANT'S COMPOST V - CV	3.982	c	4.428	c	3.990	c	4.228	c
<i>Trichoderma</i> sp. - TW2	3.654	d	3.908	d	3.641	d	3.898	e
Untreated control - C	3.615	d	3.871	d	3.617	d	3.879	e
<i>P(F) Treat</i>	<i>0.000</i>							
<i>P (F) Year</i>	<i>0.000</i>							
<i>P(F) Treat*Year</i>	<i>ns</i>							
<i>P(F) Rhizo*Bulk</i>	<i>0.000</i>							
<i>Pseudomonas (logcopy DNA⁻¹)</i>								
Serenade Max - SM	4.295	b	3.928	c	4.337	b	3.902	c
Remedier - RM	4.249	b	4.109	c	4.194	b	4.148	c
<i>P. putida</i> - Pp	4.599	a	4.506	a	4.921	a	4.781	a
ANT'S COMPOST M - CM	4.781	a	4.301	b	4.837	a	4.515	b
ANT'S COMPOST V - CV	4.292	b	3.566	d	3.880	c	4.554	b
<i>Trichoderma</i> sp. - TW2	3.654	c	3.908	c	3.735	c	3.681	d
Untreated control - C	3.495	c	3.234	e	3.435	d	3.252	e
<i>P(F) Treat</i>	<i>0.000</i>							
<i>P (F) Year</i>	<i>0.000</i>							
<i>P(F) Treat*Year</i>	<i>ns</i>							
<i>P(F) Rhizo*Bulk</i>	<i>0.000</i>							
<i>Trichoderma (logcopy DNA⁻¹)</i>								
Serenade Max - SM	3.988	d	2.928	c	3.936	d	2.902	d
Remedier - RM	4.249	c	3.109	bc	4.345	d	3.252	c
<i>P. putida</i> - Pp	3.989	cd	3.320	b	3.992	d	3.507	b
ANT'S COMPOST M - CM	4.905	b	3.337	b	5.001	b	4.621	a
ANT'S COMPOST V - CV	4.292	c	3.566	a	4.775	c	4.668	a
<i>Trichoderma</i> sp. - TW2	5.676	a	3.433	b	5.658	a	4.418	b
Untreated control - C	3.327	e	2.384	d	4.207	d	3.293	d
<i>P(F) Treat</i>	<i>0.000</i>							
<i>P (F) Year</i>	<i>0.000</i>							
<i>P(F) Treat*Year</i>	<i>ns</i>							
<i>P(F) Rhizo*Bulk</i>	<i>0.000</i>							

946
 947 Values represent marginal means
 948 *Serenade max – SM: *Bacillus subtilis* ; Remedier – RM : *Trichoderma asperellum* + *T. gamsii*; *Pseudomonas putida* – Pp; ANT'S COMPOST M –
 949 CM: Green compost + *Trichoderma* TW2; ANT'S COMPOST V – CV: Green compost; *Trichoderma* sp. – TW2
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Table 8 Pearson's correlation coefficient between microbial gene abundance, disease severity (DS) and potential nitrification activity (PNA) assessed in rhizosphere (rhizo) and bulk soil samples

Microbial abundance	DS		PNA	
	rhizo	bulk soil	rhizo	bulk soil
FOL	0.73***	0.57***	ns	-0.7***
16S Bacteria	ns	0.23		
16S Archaea	-0.35	-0.24		
18S Fungi	ns	-0.39**		
AOB	-0.45**	0.75***	-0.31	-0.5***
AOA	-0.67***	-0.65***	-0.5***	0.87***
<i>Bacillus</i>	-0.38**	-0.38*		
<i>Pseudomonas</i>	-0.44***	-0.35*		
<i>Trichoderma</i>	ns	-0.65***		
<i>chiA</i> gene	ns	-0.46**		
<i>phlD</i> gene	-0.7***	-0.5**		
<i>hcnAB</i> gene	-0.67***	-0.76**		
PNA	0.253	-0.6***		

Significance levels: not significant-ns: $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

974 Table 9 Pearson’s correlation coefficient between, *Bacillus*, *Pseudomonas*, *Trichoderma*, FOL
 975 population gene abundance and chemical properties assessed in rhizosphere (rhizo) and bulk soil
 976 samples
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Property	<i>Bacillus</i>		<i>Pseudomonas</i>		<i>Trichoderma</i>		FOL	
	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil
pH	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TN	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
NH ₄ ⁺	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
NO ₃ ⁻	-0.5	<i>ns</i>	<i>ns</i>	0.60**	-0.57**	<i>ns</i>	<i>ns</i>	<i>ns</i>
PT	-0.80***	-0.50**	0.60***	<i>ns</i>	0.6**	<i>ns</i>	-0.5**	-0.76**
AP	0.50**	-0.60**	0.75***	0.40*	0.55**	0.60**	0.565**	0.438*
TOC	-0.6**	0.4 *	-0.70***	0.60**	-0.75***	0.76***	0.657***	0.543**

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 980 Significance levels: not significant-ns: p>0.05; *p<0.05; **p<0.01; ***p<0.001
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